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Environmental Solutions to Problems Caused by Arthropods of Public Health Importance in the Jordan Rift Valley and the Gaza Strip

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Section I

Background

Seasonal outbreaks of flies and mosquitoes have progressively emerged over the past several years in the Southern Dead Sea Basin (SDSB) situated in the Jordan Rift Valley (JRV) and the Gaza Strip, significantly inhibiting economic growth and adversely affecting the quality of life. These massive outbreaks have been related to environmental disturbances, which are largely the result of faulty drainage, waste and agricultural management practices.

Houseflies (*Musca domestica*)

Massive housefly population outbreaks constitute a serious nuisance to tourists, inhabitants, agricultural laborers and factory workers in the SDSB and Gaza strip regions. In addition to being a nuisance pest, houseflies have been implicated in the transmission of more than 20 human and animal diseases. Mastitis, pinkeye, anthrax, typhoid fever, amoebic dysentery, tuberculosis, cholera, Newcastle disease and salmonella are some of the diseases affecting man and animals that flies can transmit. The housefly is also the intermediate host for some roundworms and tapeworms of poultry.

The mainstay of any housefly control program is sanitation and the elimination of those conditions which permit breeding and reproduction (West, 1951). Agricultural fields have been identified as the major source of fly breeding in the SDBS while poultry farms and municipal dump sites are the major sources in the Gaza Strip region (Annual Reports, 1995-1999). Each situation requires a unique management policy based on scientific findings and adaptation of environmentally sound control measures to the conditions prevailing in the study area.

Mosquitoes

During the first phase of this research project 16 mosquito species were identified (Annual Reports 1995 - 1999) four of these species are considered to be of public health importance: *Anopheles sergentii* and *An. superpictus* are potential malaria vectors. *Culex pipiens,* the domestic nuisance mosquito and *Cx. perexiguus* are the vectors of West Nile fever and Sindbis viruses in the Eastern Mediterranean region. In Egypt *Cx. pipiens* has also been incriminated as the major vector of the deadly Rift Valley virus. It has been found to be the most dominant nuisance mosquito in the SDSB as in the rest of Israel. *Cx. pipiens* bites at night while the second most abundant mosquito the "salt marsh mosquito"- *Aedes caspius* is a very disturbing daytime biter.

An integrated biological mosquito control (IBC) program based on utilization of all available techniques including: physical source reduction, utilization of the environmentally friendly biological control agent *Bacillus thuringiensis* subsp. *israelensis* (Bti) and augmentation of natural enemies (*Aphanius dispar, Anax imperator* and others), was partially developed in the first three years of this project . During this time a thorough mapping of all breeding sites was carried out and routine surveillance of larval and adult mosquitoes made (Annual Reports 1995-1999).

This report summarizes the results of our activities carried out during the 2000 season.

A. Research Objectives

The overall scientific objective of this project is to determine the necessary environmental interventions to be effectively directed against the proximal cause of the enormous outbreaks of mosquitoes and flies that periodically infest the inhabitants and visiting tourists. The proposed regional scientific effort is designed to contribute to the well-being of the residents of the affected areas, to promote public health, tourism and agriculture by helping to alleviate these problems and serve as a model for other countries in the region.

I Houseflies (*Musca domestica*)

The specific research objectives over the past year were to: 1) continue routine monitoring as a basis for measuring the effect of control intervention, 2) develop a solid waste management scheme, 3) develop fertilization practices in agriculture designed to reduce or eliminate fly breeding and 4) provide recommendations and protocol for sustainable integrated fly control in the region.

II Mosquitoes

The specific research objectives over the past year were to: facilitate and supervise control intervention where necessary, continue developing protocols for the local municipality, continue routine adult mosquito monitoring utilizing CDC traps, scout all potential breeding sources for presence of larvae and to determine the effects of salinity on oviposition preference and larval development at the South Dead Sea basin.

B. Research Accomplishments

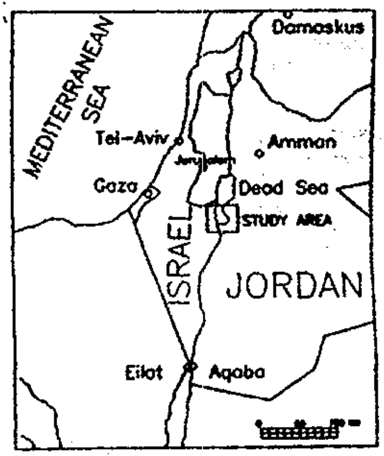
I Houseflies

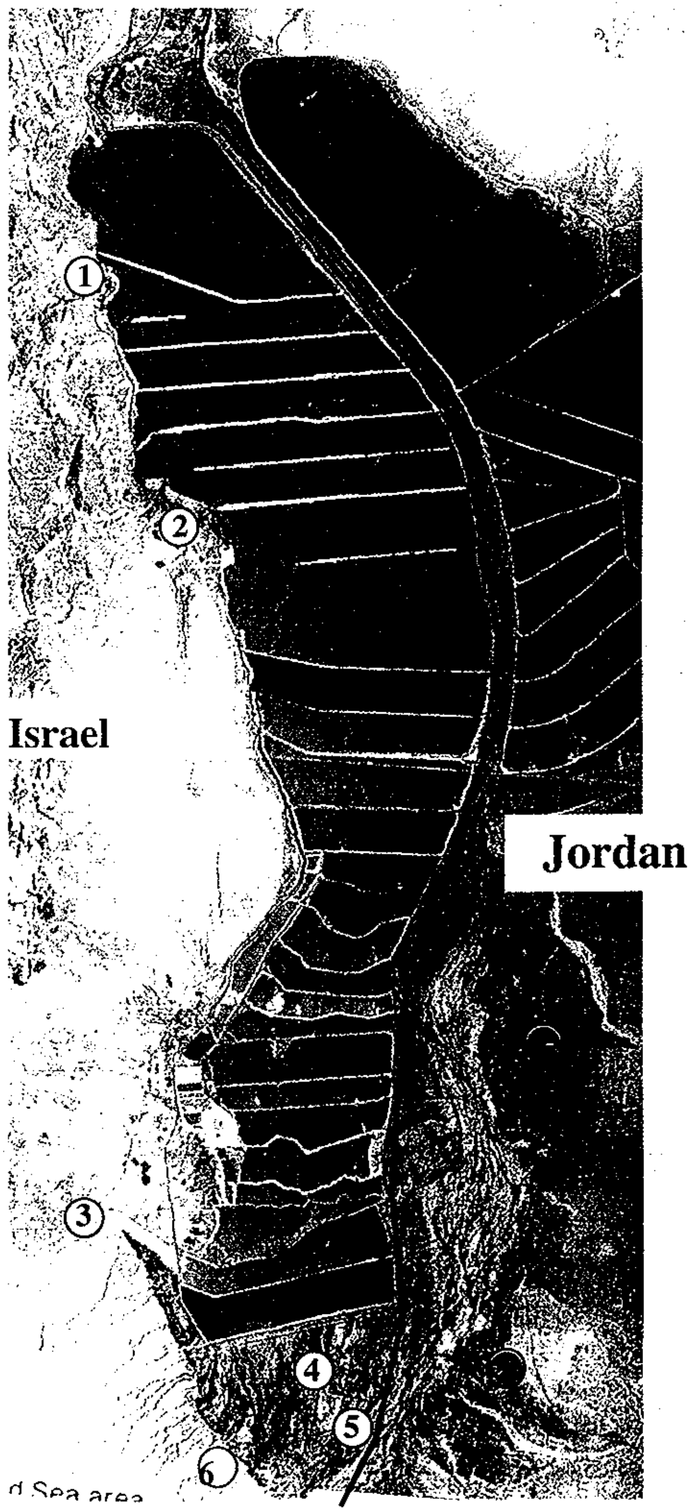
During the course of 2000 the following activities were conducted: 1) spatio-temporal distributions of adult housefly population densities and sex ratios were determined; 2) a capture-release-recapture experiment, initiated and conducted in Jordan was jointly monitored here; 3) results of the pilot compost process was analysed and plans for a pilot plant developed; 4) data amassed over the past 5 years were collated and recommendations for a regional integrated fly management program made.

a. Monitoring

During the year 2000 we continued our ongoing monitoring program. An array of eighteen standard cone bait traps was set up at 5 selected sites in the study area (fig. 1). This year the Compost site was added in order to monitor fly populations adjacent to the experimental pilot compost plant in the agricultural area of Neot Hakikar. Three traps, ca. 15 m from each other were positioned at each of the sites (4 traps at compost). Traps were monitored every week from mid April to end of November. Trapped flies were placed into marked plastic bags and brought to the laboratory for processing. The number of flies was estimated and a sample of 150-200 flies per cage was taken to determine sex ratio.

Figure 1. Monitoring array for adult houseflies in Southern Dead Sea Basin, 2000. Agricultural areas in Jordan (Safi and Fifa) and in the south around monitoring sites 4, 5 and 6. 1-6 are sampling sites; 7 and 8 are agricultural settlements in Jordan.





1. Hotel Region = **HO**

2. Hemar reservoir = **MH**

3. En Tamar Junction = **TJ**

4. Compost = **Comp**

5. East = **East**

6. Neot Hakikar = **NH**

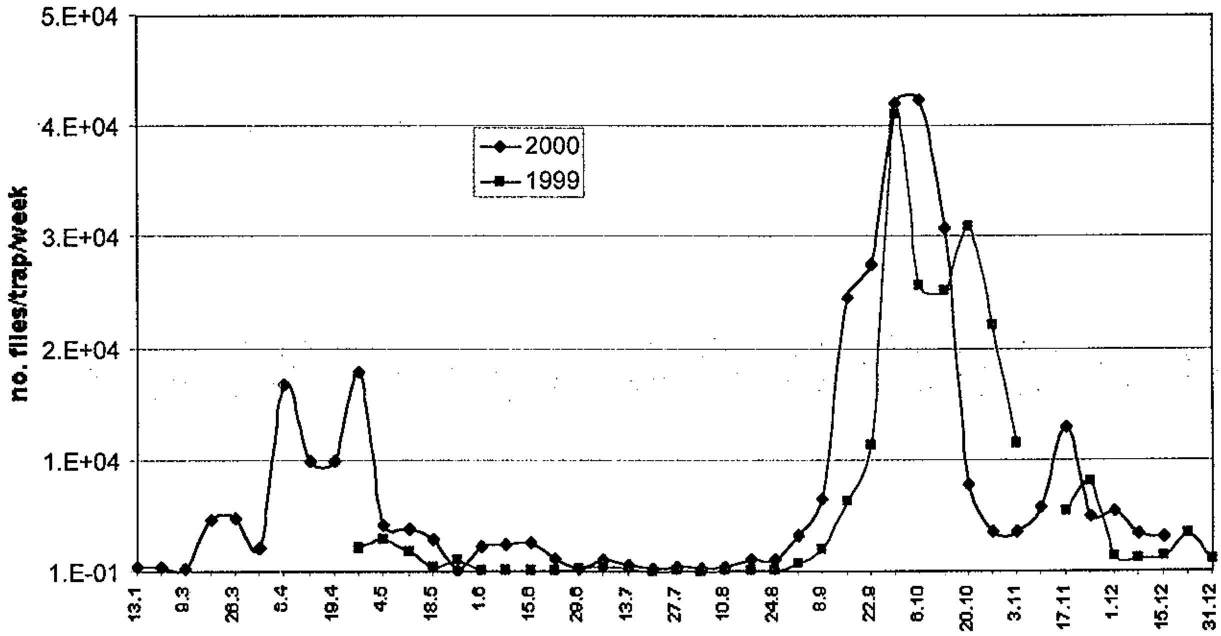
7. Fifa

8. Safi

b. Temporal distribution

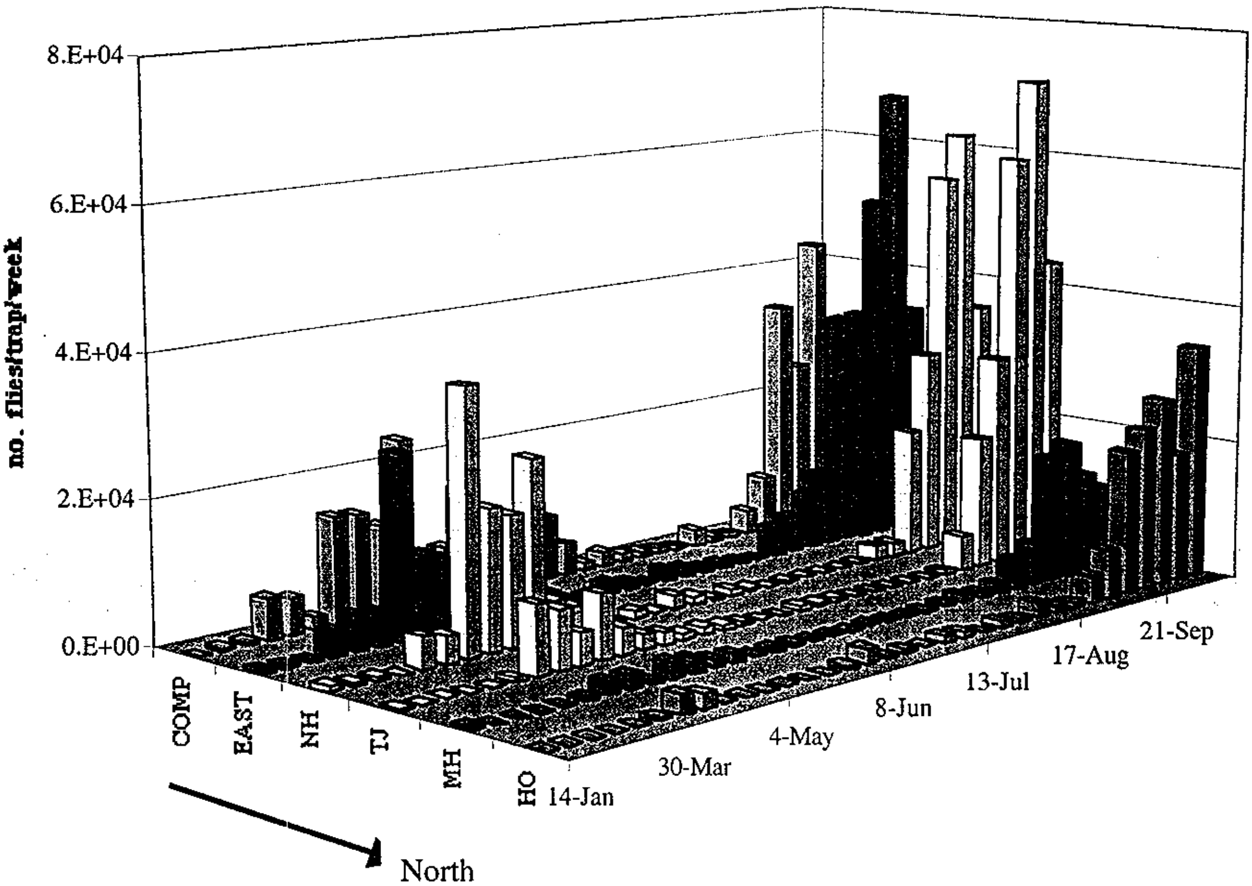
This year (as in previous years) two seasonal peaks of adult flies were recorded in the spring and fall (fig. 2). The spring peak occurred in April-June in the southern part of the study area and the fall peak occurred in mid-September. Adult populations continued being a nuisance till mid November. Weekly trap catches reached levels over 40 thousand flies per week per trap.

Figure 2: Temporal distribution of *Musca domestica* in the Southern Dead Sea Basin, 2000.



Spring populations were concentrated in the southern part of the research area while fall populations were more widely distributed throughout basin (fig. 3).

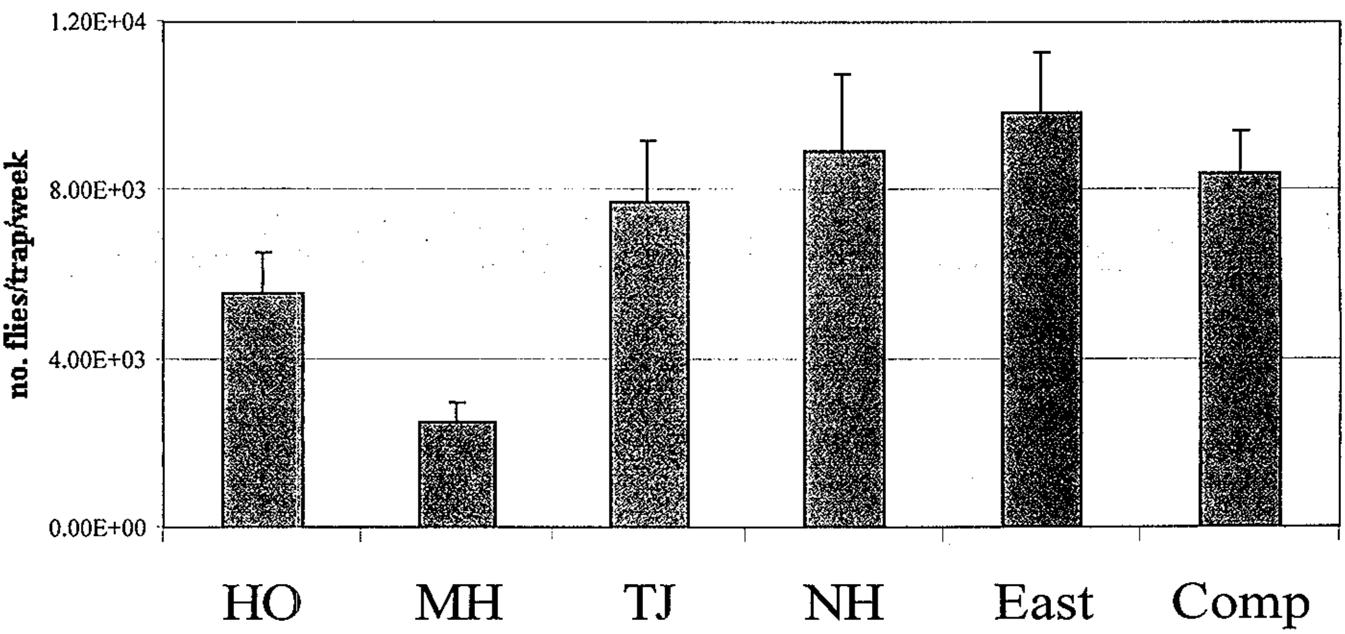
Figure 3: Spatiotemporal distribution of *Musca domestica* in the Southern Dead Sea Basin, 2000. (COMP = Compost, NH = Neot Hakikar, TJ = Tamar Junction, MH = Ma'agar Hemar, HO = Hotels). COMP, NH and TJ are in the south and HO is in the north, MH is in the middle.



c. Spatial distribution

Fly population densities were greater in the southern part of the research area; Neot Kikar (NH), East and Compost; with decreasing densities toward the north Tamar Junction (TJ), Heimar reservoir (MH) and Hotel region (HO). A characteristic peak around the hotel area was recorded this year, as in previous years, indicating that the local source of breeding at the En Bokek water treatment facility has not been entirely eliminated.

Figure 4: Spatial distribution of *Musca domestica* in the Southern Dead Sea Basin, 2000.



d. Sex ratio

Sex ratios (f/m) were determined on all sampling occasions from mid May to the end of November. Results were analysed using one-way ANOVA with SuperAnova® software to determine the effect of trap site (geographical location) and sampling date (time of season) on sex ratios. Significant differences in sex ratios were found both between sampling sites and sampling dates. Sex ratios at Tamar Junction were characteristically low. Sites in the southeast part of the research area were relatively high with lower ratios in the northern sites (HO and MH). Temporal variation in sex ratio occurred between spring, summer and fall populations. High f/m ratios were recorded in early spring with a rapid drop during the peak spring season, increasing again during the summer when population densities are low and again dropping during the peak fall season.

Figure 5: Sex ratio of adult female/male fly populations analysed by site.

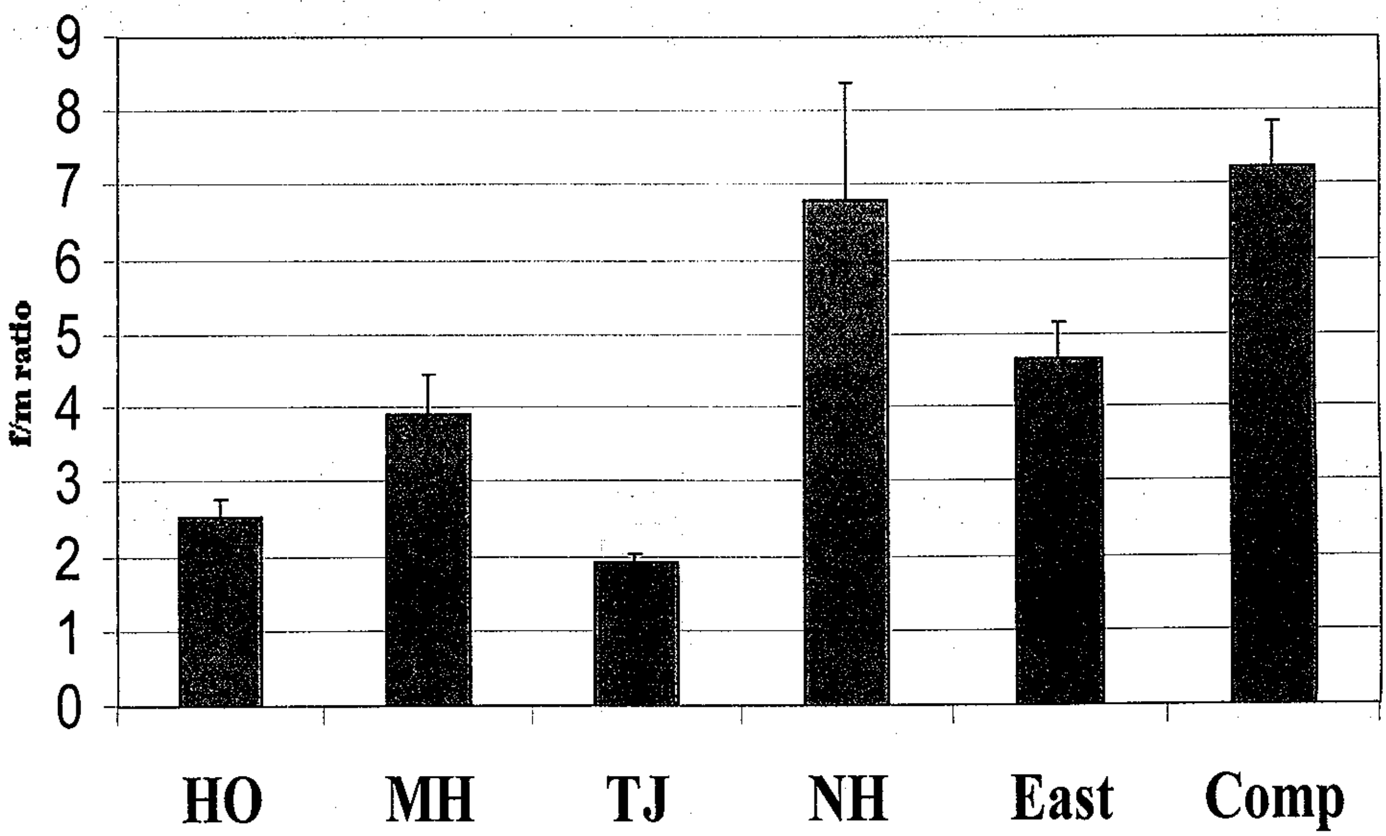
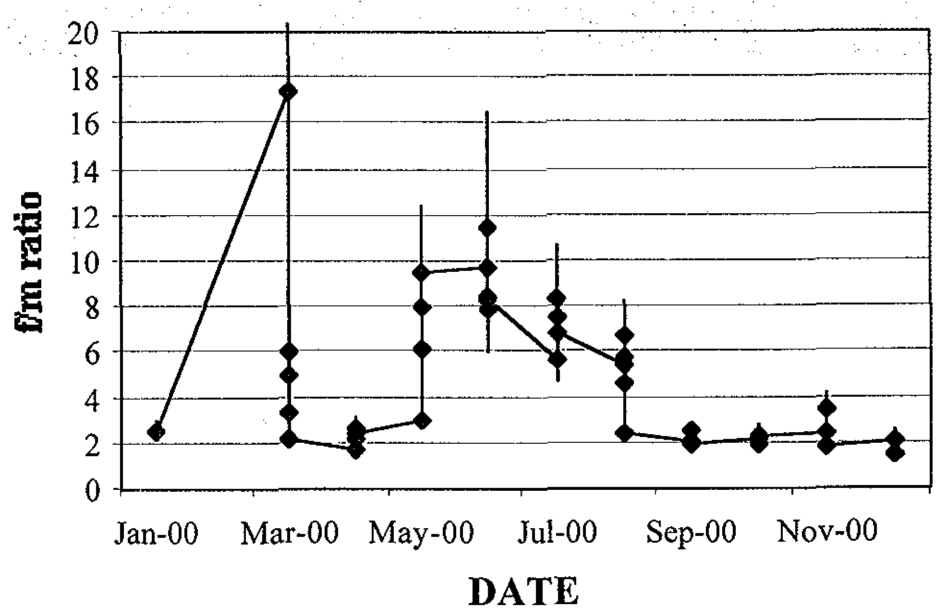
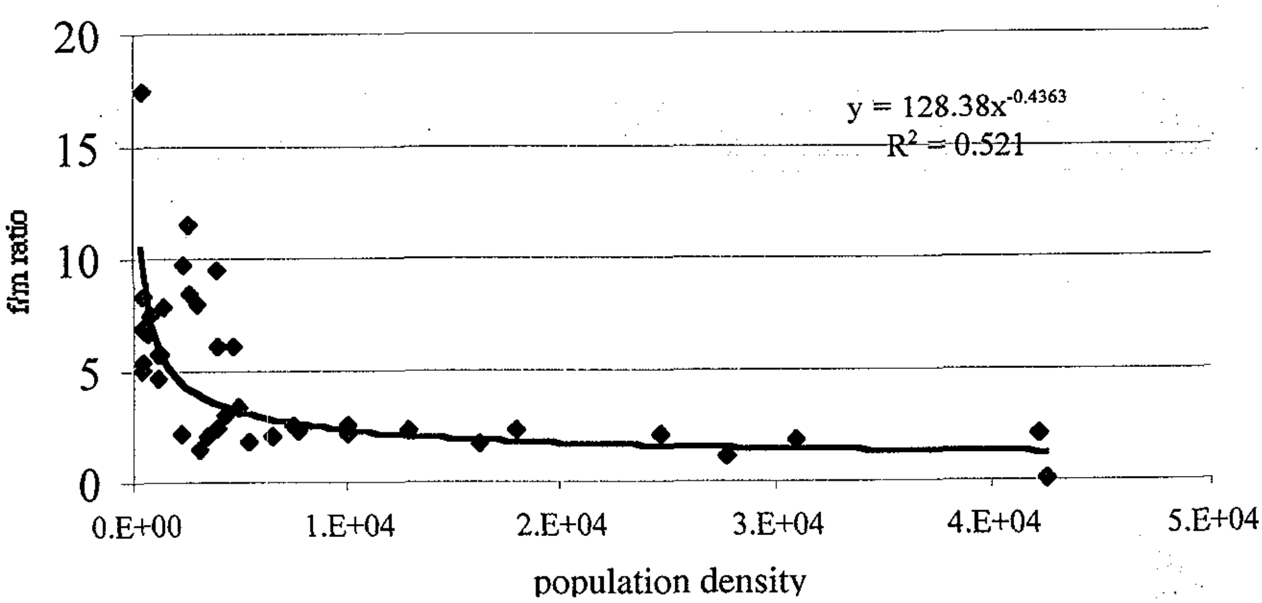


Figure 6: Sex ratio of adult female/male fly populations analysed by date.



A general relation between sex ratio and population density was obtained. Large population densities were accompanied by lower f/m ratios whereas higher f/m ratios were characteristic of lower densities.

Figure 7. Sex ratio as a function of population density.



e. Dispersal experiment - initiated in Jordan

Our colleagues in Jordan conducted a capture-release experiment on 22.9.00. They requested our assistance in this experiment by asking us to set up some collection traps on the Dead Sea Works dikes in the vicinity of their release point in order to obtain data on flies dispersing east over the international border. Release and capture were synchronized through several email messages and telephone conversations. In this experiment, flies were released at some point on dike 5 of the Arab Potash Works. We placed 7 collection traps, arranged 0.5 km apart from each other, on the eastern border of the Dead Sea Works dikes in the approximate vicinity of dike 5 of the Arab Potash Company, in a T configuration (fig. 8). Traps were prepared and supplied with standard yeast bait + water 3 days before release in order to allow maximum trapping efficacy at the time of release. Two collections were made at 10:30-10:50 and 11:30-11:50. Flies were taken back to the laboratory and examined with an UV light and the number of marked flies recorded (table 1). We did not conduct any dispersal experiments in Israel this year, instead we have analysed data collected over the past three years, which we intend to publish in thenear future.

Figure 8. Map of collection sites on the Dead Sea Works dikes. Flies released in Jordan. 22.9.00

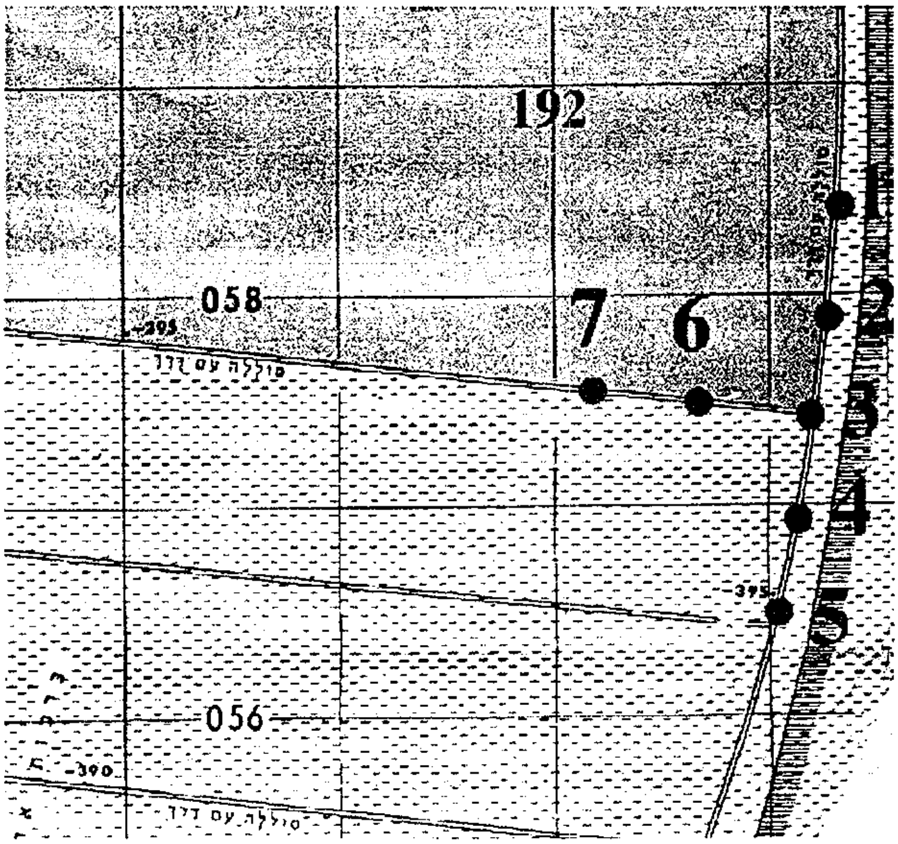


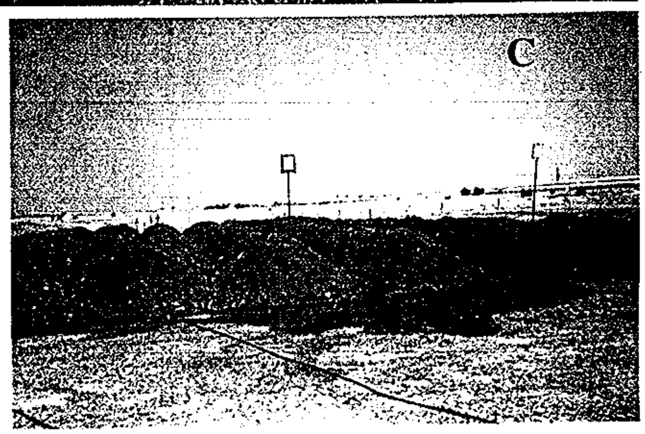
Table 1. Number of flies recaptured in Israel from Jordanian release, 22.9.00.

Time	**Trap**	**Colored flies (red)**	**Non-colored**
10:30-10:50 22.09.00	1	5	492
	2	5	414
	3	18	1370
	4	19	720
	5	33	1160
	6	6	690
	7	4	960
11:30-11:50 22.09.00	1	4	226
	2	5	224
	3	8	460
	4	3	259
	5	3	325
	6	3	164
	7	2	420

f. Compost

During the current reporting year, approximately 5,000 m^3 raw agricultural solid waste was processed into ca. 900 m^3 enriched compost, recycled back into the agricultural fields.

Figure 9. Pilot thermophilic compost plant. Raw agricultural waste and final enriched compost recycled into the agricultural fields.



A. Dumping solid waste at compost site
B. Solid waste before composting
C. Final product, high quality compost

Solid Waste Management Scheme

During the current reporting year we have developed a solid waste management scheme with the primary objective of establishing a pilot plant in the agricultural area of the Southern Dead Sea Basin. A potential site for this pilot plant was determined in coordination with the local governing municipalities and farmers in the region (fig. 10).

Figure 10. Agricultural region of the Southern Dead Sea Basin (500 ha) with planned site for pilot compost plant (yellow area).

Municipal Agricultural Organic Waste Treatment plant jointly with Ministry of Agriculture, Agricultural Research Organization (ARO), gilat coordinated by Dr. Leah Tsror.

Overall objective	Waste management program in the Jordan Rift Valley		
Project purpose	**Pilot organic waste recycling facility in the Southern Dead Sea Basin (SDSB)**		
Components	**Secondary objectives**	**Parameters to be tested**	**Records to be maintained**
Results Thermophilic and vermiculture processes	1. Reduced fly populations	Monitoring population dynamics	Records – BGU
	2. Phytosanitary - clean fields and compost	Diagnostic pathogen tests	Records – ARO
	3. Reduce landfill waste	Increased land use (reduce land waste)	Municipal records
	4. Clean groundwater	Water sample analysis	DSW laboratory records
	5. Local supply of enriched fertilizer	Metric ton fertilizer locally produced	Municipal records and farmer reports
Activities Thermophilic and vermiculture processes	1. Collect organic waste - Remove diseased leaves and plant parts from field	Quantity of waste collected	Municipal records
	2. Bring waste to compost facility	Quantity of waste received	Facility log data
	4. Shred organic waste	Hours of shredding	Facility log data
	5.Compost process (three components above)	MT compost produced	Facility log data
	6. Recycle drain water	Volume of drainwater	Facility log data
	7. Add pathogen antagonists	Reduction in soil diseases	Phytopathologist's test
	8. Add locally available soil nutrients (e.g., potash from Dead Sea Works, Ltd. (DSW))	Effect on yield of locally grown crops	Experiments and farmers' records

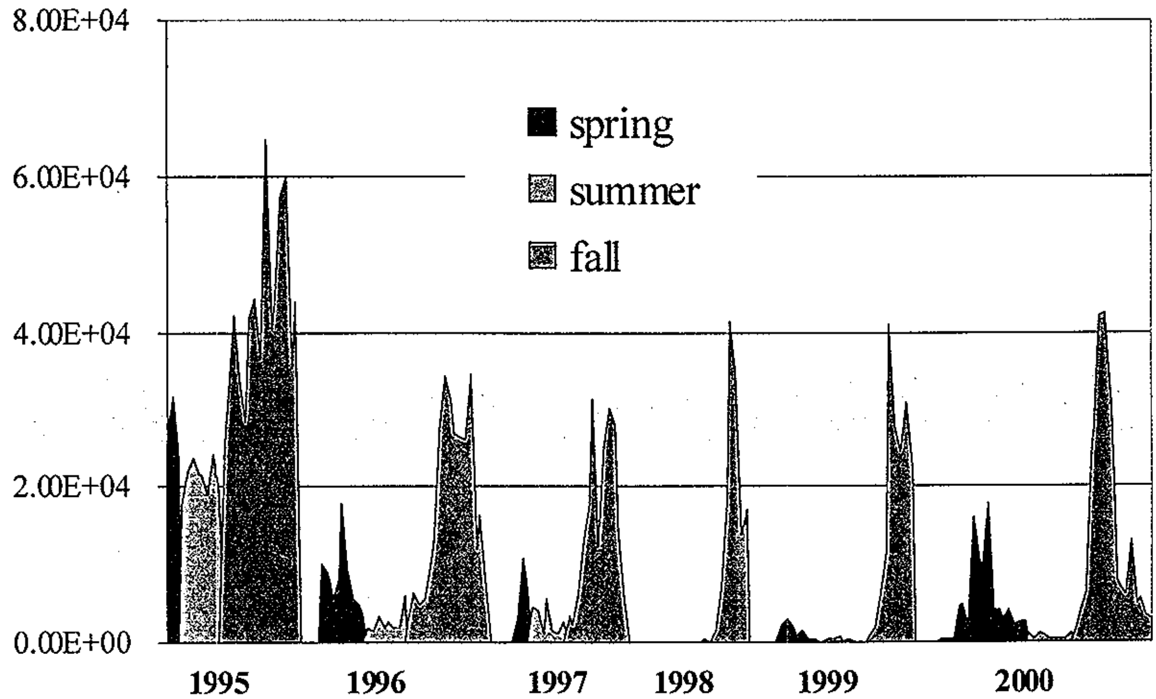
g. Musca domestica (5-year summary of results)

Spatiotemporal distributions of adult houseflies were determined over a 5-year period from 1995-2000.

All data were entered into a single database containing the following fields:

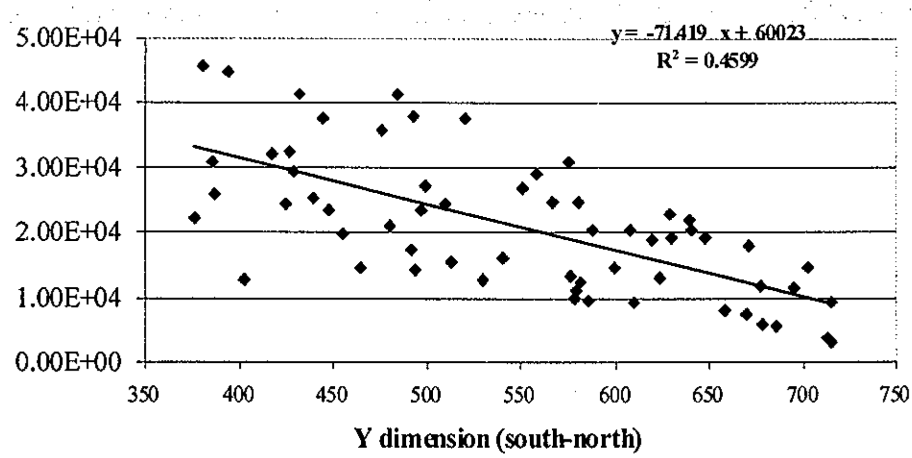
Temporal distributions described by massive fall peaks and minor spring speaks with very little activity in the summer repeated itself throughout the study (fig).

Figure 11. Temporal distribution of fly populations in the Southern Dead Sea Basin, 1995-2000



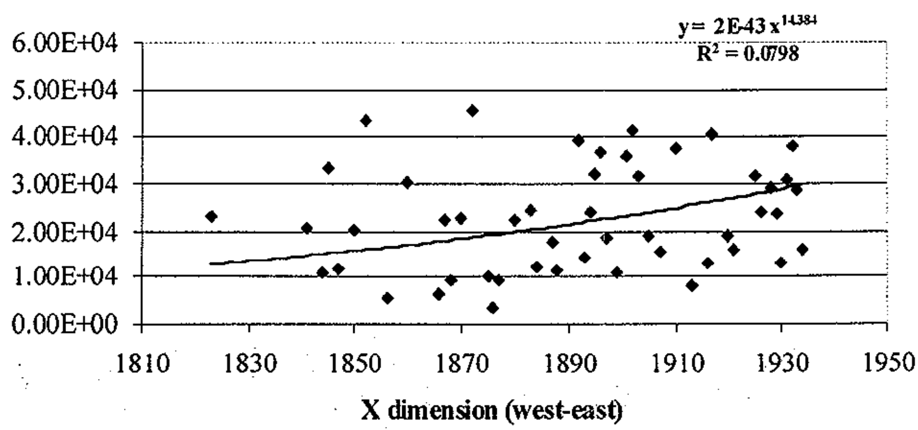
Adult fly populations were denser in the southern part of the study area with decreasing densities to the north (figure 12).

Figure 12. Bivariate scatterplot of the number of adult flies against X (northing) direction (SDSB, 1995-2000).



Adult fly populations were denser in the eastern part of the study area with decreasing densities to the west (figure 13).

Figure 13. Bivariate scatterplot of the number of adult flies against Y (easting) direction (SDSB, 1995-2000).



II Mosquitoes

During the current reporting year we have conducted the following activities:

a) Principle breeding sites

b) Monitoring adult mosquitoes.

c) Artificial pool experiments testing preference to varying salt concentrations

d) Integrated biological control using Bti

a. Principal breeding sites of mosquitoes in the Southern Dead Sea Basin

Five principal types of mosquito habitats in which mosquitoes complete their life cycle in the Southern Dead Sea Region are as follows:

1) Salt marshes with high aquifer level and fluctuating ion concentration around 30 g/l Cl^- in a general area of 40 ha.

2) Drainage canals 15 km in length and with a general area of 3 ha and fluctuating ion concentration around 5 g/l Cl^-.

3) Springs with variable salinity levels between 2-5 g/l Cl^-.

4) Sewage treatment pools

5) Zin, an artificial reservoir with an area of ca. 25 ha created from the flood waters originating from the occasional flash floods along the Zin river watershed. Salinity levels in the water were measured before floods at a level of 52.9 g/l Cl^- and after floods at 0.5 g/l Cl^- representing a dilution of 100 fold. The artificial reservoir of clear water provides a habitat for a large variety of aquatic organisms and place for water fowl to gather.

6) Heimar reservoir was constructed in 1991 in order to collect flood water from the Heimar, Lot and Prazim rivers and to prevent entrance of water to the Dead Sea Works evaporation pans. The maximum capacity is 3 million cubic meters and is densely populated with reeds and tamarisk. This reservoir was a breeding habitat during the winter of 1997/8 when flooding occurred and again in the winter of 2000 when several mosquito species were found breeding at relatively high population densities.

Mosquito Species

Ten species of mosquitoes were found at the different sampling sites in the SDSB. Larval abundance was estimated on a scale of 1 to 5 (1 for the least common and 5 for the most prevalent) (see list below). Some species were only found in few localities though in high numbers. *Cx. pipiens* and *Cx. perexiguus* were for instance found in the fish pond in extremely high numbers.

Aedes caspius Pallas (5)
Anopheles multicolor Cambouliu (2)
Anopheles sergentii Theobald (4)
Culex deserticola Kirkpatrick (1)
Culex perexiguus Theobald (3)
Culex pipiens Linnaeus. (4)
Culex pusillus Macquart (2)
Culex sinaiticus Kirkpatrick (2)
Culex theileri Theobald (1)
Culiseta longiareolata Macquart (3)

b. Monitoring adult mosquitoes.

Adult mosquito populations were monitored during the current reporting year from March-December in 4-5 permanent sampling sites (fig. 14). Mosquitoes were trapped once every 2-3 weeks with the aid of dry ice charged CDC light traps. Trap catches were brought to the lab for processing where mosquitoes were identified to species and counted. A total of twelve species were recorded during the current reporting year. *Culex pipiens* constituted the most prevalent species constituting 95% of the trapped mosquito population. The second most prevalent species was *Ae.caspius* representing 2.6% of the total population (fig. 15).

Figure 14. Sites (marked by X) chosen for CDC light trapping of adult mosquitoes in the SDSB, 1999. A drainage canal of the newly constructed fish ponds constitutes a new breeding habitat, which till 1999 did not exist.

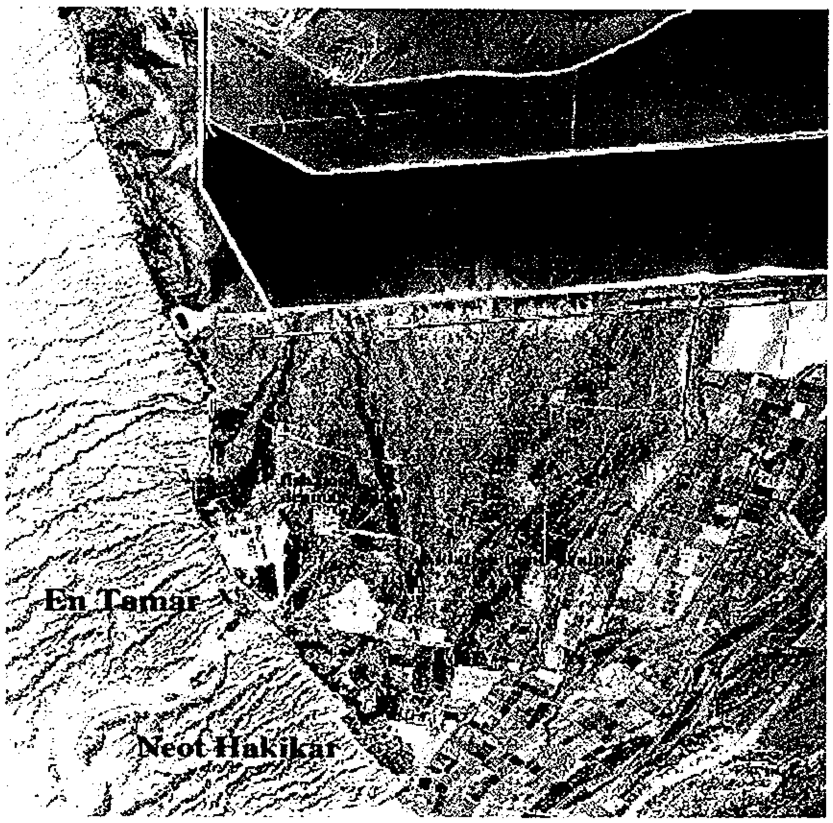
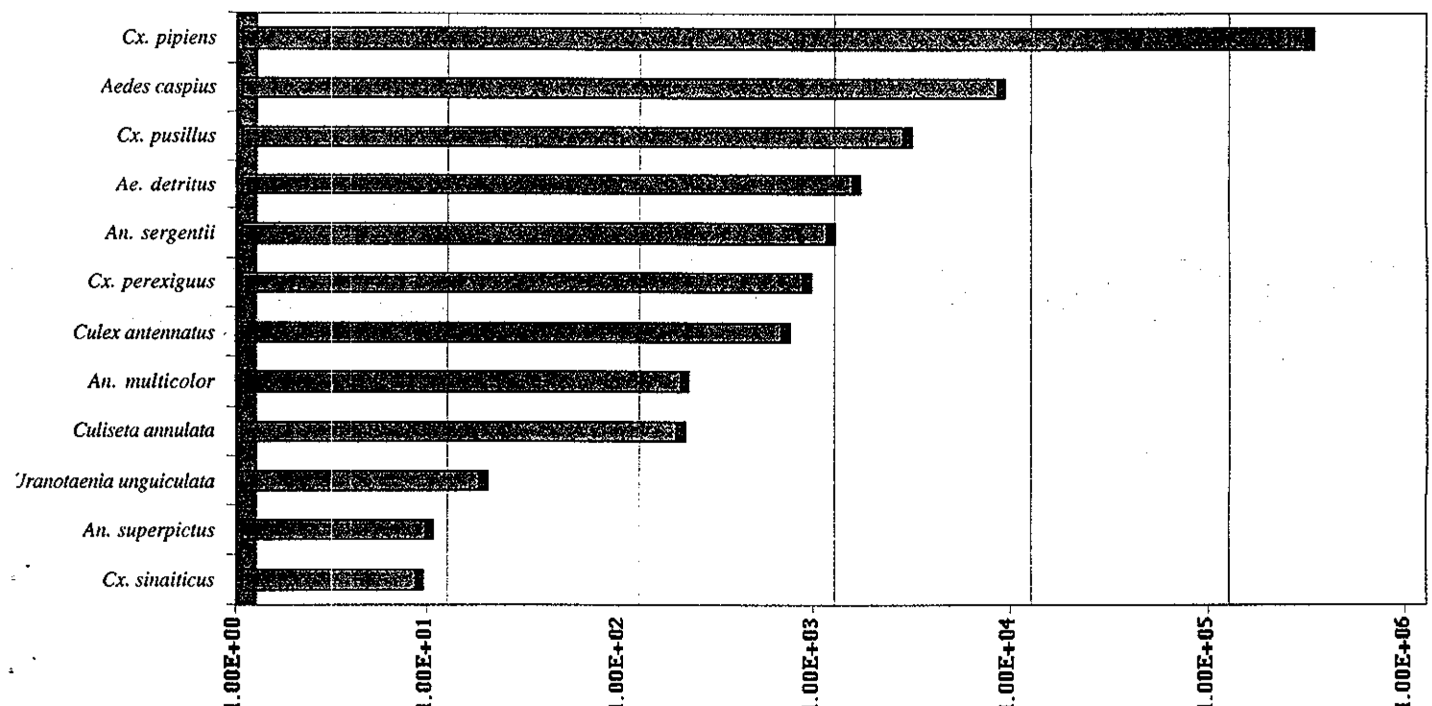


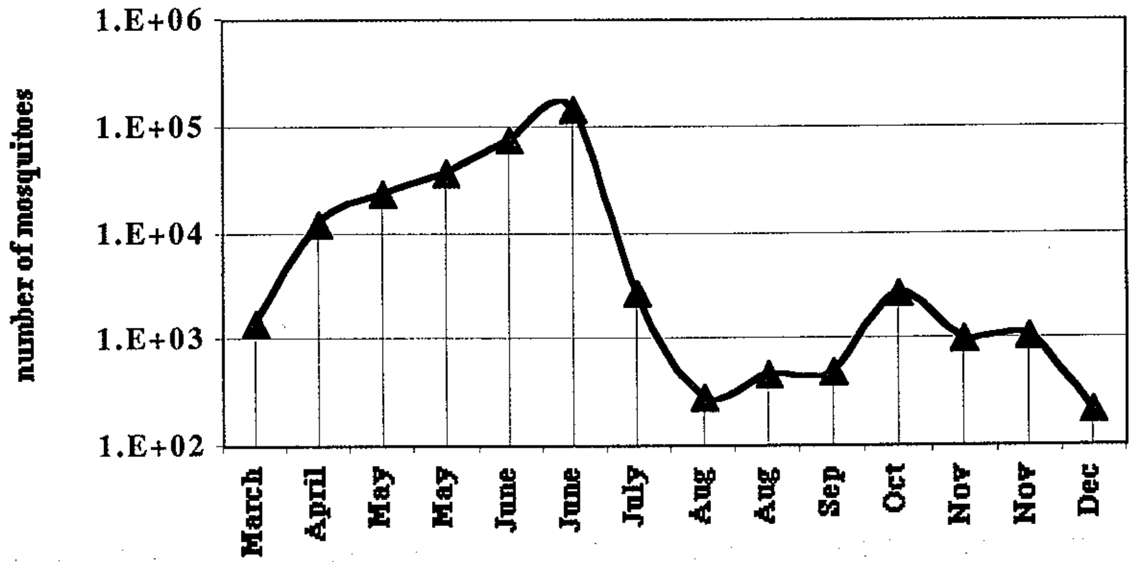
Figure 15. Species composition of mosquitoes in Southern Dead Sea Basin, 2000.



Temporal and spatial distributions

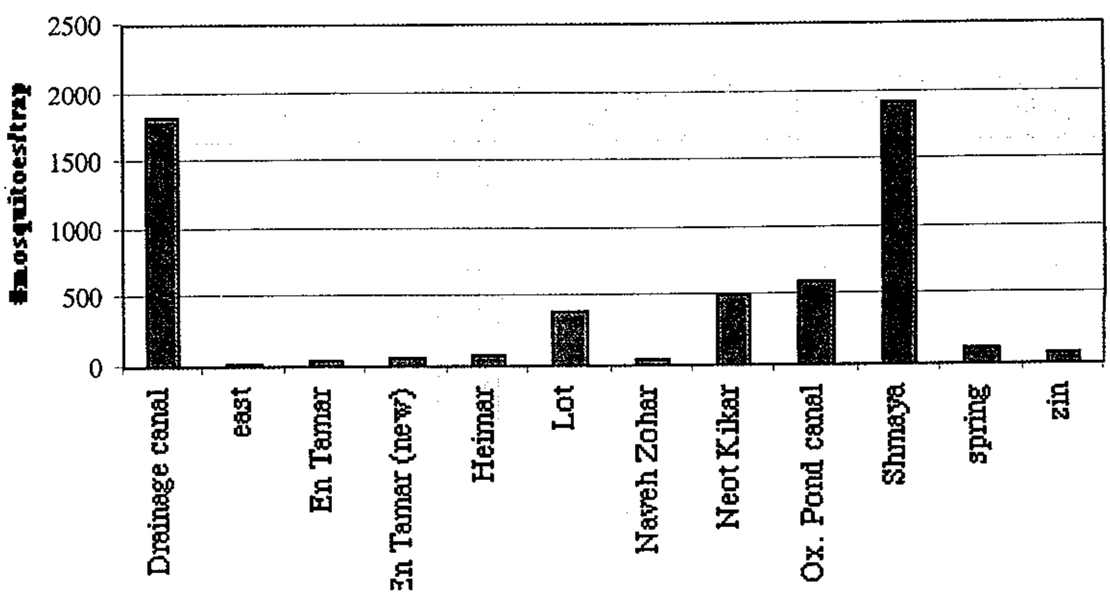
Seasonal dynamics were described by a major peak in the spring and a lower peak in the fall (fig. 16).

Figure 16. Seasonal dynamics of mosquitoes in the Dead Sea Basin, 2000.



The spatial distribution of adult mosquitoes may be described as highly aggregative, with high densities near breeding sites and lower densities at points further removed from the breeding sites (fig. 17)

Figure 17. Spatial distribution of adult mosquitoes in Southern Dead Sea Basin, 2000.



c. Oviposition preference and larval development as function of salinity

Because of the prevalence of halotrophic mosquito species in the South Dead Sea Basin (SDSB) and the phenomenon of relatively high salt ions concentrations in many water sources, it is hypothesized that water salinity is an important factor in limiting the distribution of mosquito species in the area. Knowledge about this factor may be a useful tool in studying the distribution of mosquito species (Foley and Bryan 1999). Salinity may effect ovipositional site preference as well as larval and pupal development. Ovipositional preference may also be influenced by other factors such as color of the water, size and shape of the water body (Yap et al. 1995), or the presence of predators (Blaustein 1998). The relation between salinity and oviposition preference in halotrophic species is not simple, and species that are physiologically capable of surviving at high concentrations of salt may be found only in fresh water due to ovipositional preference (Grueber and Bradley 1994), while others avoid ovipositing in fresh water (Roberts and Irving-Bell 1997). Oviposition preference does, therefore, not necessarily correspond with the ideal concentration for larval development (Roberts 1996).

The purpose of this study was to determine the effects of salinity on oviposition preference and larval development at the South Dead Sea basin.

Materials and methods

Location: The experimental site was located about 1 km. east of the new settlement En-Tamar in the Southern Dead Sea Basin.

Experimental design: Randomized blocks with 4 treatments (salt concentrations) and 6 replications.

Treatments: 4 concentrations of NaCl:

1. 0 gr./l
2. 10 gr./l
3. 20 gr./l

4. 30 gr./l

(0 gr./l is tap water and 3 gr./l is approximately seawater).

Materials: Green plastic containers (50x40x20) cm were placed in depressions in the soil such that the top of the container was at soil level and filled with 35 liters of tap water. Salt (NaCl) concentrations were achieved by adding the appropriate amount of table salt and stirring till completely dissolved.10 grams of rabbit pellets (organic matter) was added to each pool, and the pools where shaded to reduce evaporation.

Sampling for aquatic organisms: Samples were taken every 3 days by sweeping the pool with a 8x15 cm. aquarium net. All samples were placed in containers and brought to the laboratory for processing. Mosquitoes were identified to species and categorized as LI+LII, LIII+LIV and pupae. Associated aquatic organisms were also identified and recorded.

Sampling for salinity: Salinity was measured using a YSI model 33 salinity meter. Distilled water was added to replace the evaporated water and maintain the desired salinity level.

Duration: The experiment lasted 50 days from April-May 2001 coinciding with peak mosquito activity in the area (Margalit et al. 1988).

Statistical analysis: The results were analyzed using repeated measures ANOVA, the repeated measure is the 15 sampling dates. Mean separation was carried out using least significant difference (LSD) at the P=0.05 level of significance.

Results

Mosquitoes:

Effect of salinity on species abundance:

10 different *CULICIDAE* species were recorded during the experimental period (table 2). The least number of species (statistically significant at P=0.05) was recorded at the highest salinity level (table 3).

Culex pipiens which comprises 95% of the adult mosquito population caught in CDC light traps (Margalit 1997), appeared in only 5 samples and only at 0 salinity (tap-water). Although this data could not be statistically analysed it reinforces the general observation that *C. pipiens* is a distinctive fresh water species (Clemens 1992).

Effect of salinity on presence and abundance of mosquito developmental stage categories **(LI+LII, LIII+LIV, and pupae)**

Two mosquito species, *Aedes caspius* and *Anopheles multicolor* were statistically analyzed.

Aedes caspius

LI+LII: The mean number of larvae in category LI+LII was greatest in pools containing 0 and 10 gr./l, and the mean was significantly lower in pools containing 30 gr./l (table 4). Differences in mean number of larvae in the early stages of development provide some indication of oviposition preference.

LIII+LIV: No significance was found in mean number of larvae in category LII+LIV between the 4 salinity levels. The mean number of larvae equilibrated between all treatments by the time that they reached latter developmental stages. This may indicate that larval development is slightly superior in higher salinities.

Pupae: The number of pupa was significantly higher at 10 gr./l than 0 and 30 gr./l (table 5). This result raises the possibility that the ideal salt concentration for development to the pupal stage is about 10 g/l.

In general: The mean number of larvae (and pupae) for this species was highest at 10 gr./l and significantly lower at 30gr./l (table 6). These results provide evidence for the wide salinity tolerance range of this species in the SDSB region (Margalit and Tahori 1985), and also provides evidence for preferential oviposition in lower salt concentrations (Metge and Hassaïne 1998).

Anopheles multicolor

LI+LII: The mean number of larvae in these stages was greatest in pool containing tap water, and the mean was significantly lower in 20 and 30 gr./l (table 7). These results may point to an oviposition preference at the lower concentrations of salinity.

LIII+LIV: At this age group the mean number of larvae was highest at 10 and 20 gr./l. Although the mean number of larvae was still significantly lower at 30 gr./l (table 8), this result may raise the possibility that higher concentrations of salt are more suited for the development of larvae from this species.

Pupae: No significant differences where found for pupae between the various concentrations. This result may be further evidence that larvae of this species develop better at higher concentrations of salt although oviposition rates were higher at the lower concentrations.

In general: The larvae of this species were also found at a large salinity range, showing a preference to lower degrees of salinity with a significant drop at 30 gr./l (table 9). This result is due to the higher presence of 1^{st} and 2^{nd} instar larvae at lower concentrations. At natural water sources, this species is known to populate a wide range of salinities and to prefer high concentrations of Sodium (Margalit and Tahori 1988), so these results are in need of further testing.

Other insect taxa: The presence of 3 other insect species associated with mosquito breeding was monitored during the time of the experiment. Larvae of Ephydridae showed a strong preference to higher degrees of salinity with a significant peek in the mean number of larvae at 30 gr./l, a drop towards the intermediate concentrations and a significant drop at 0 gr./l (table 10).

Larvae of Chyronomidae showed exactly the opposed with a peek at 0 gr./l which was significantly higher then other concentrations (table 11).. Mayfly larvae showed no significant difference between salinities.

Table 2: CULICIDAE species that oviposited at the pools during the experiment.

Species:	Number of individuals	% of total
Aedes caspius	*20830*	84.51
Anopheles multicolor	3167	12.85
Culex pipiens	311	1.26
Culex pusillus	119	0.48
Culex theileri	109	0.44
Anopheles sergentii	50	0.2
Aedes detritus	38	0.15
Cs. Longiareolata	19	0.08
Anopheles superpictus	3	0.01
Culex perexiguus	3	0.01

Table 3: Total number of CULICIDAE species at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
30	1.36	0.64	0.068	a
0	1.71	0.83	0.088	b
10	1.9	0.82	0.086	b
20	1.68	0.62	0.066	b

Table 4: Number of 1st and 2nd Instar *Aedes caspius* larvae at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
30	13.9	21.33	2.24	a
20	25.5	42.82	4.5	ab
0	37.46	59.4	6.26	b
10	43.23	43.23	68.6	b

Table 5: Number of *Aedes caspius* pupa at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
0	2.38	3.51	0.37	a
30	2.7	3.95	0.41	a
20	3.78	6.6	0.7	ab
10	7.4	19.61	2.06	b

Table 6: Number of *Aedes caspius* larvae at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
30	34.14	48.02	5.06	a
20	57.6	91.97	9.7	ab
0	58.48	76.21	8.03	ab
10	81.2	110.45	11.64	b

Table 7: Number of 1st and 2nd Instar *Anopheles multicolor* larvae at different salinities.

NaCl concentrat ion (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
30	0.92	3.1	0.32	a
20	3.08	6.91	0.72	a
10	3.72	8.21	0.86	ab
0	6.54	18.51	1.95	b

Table 8: Number of 3rd and 4th Instar *Anopheles multicolor* larvae at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
30	2.24	7.75	0.81	a
0	3.67	7.47	0.78	ab
20	5.65	9.47	0.99	b
10	5.74	12.35	1.3	b

Table 9: Number of *Anopheles multicolor* larvae at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
30	3.64	8.86	0.93	a
20	9.9	14.27	1.5	b
10	10.44	17.15	1.8	b
0	11.17	21.52	2.26	b

Table 10: Number of Ephydrid larvae at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
0	29.6	52.26	5.7	a
10	99.63	125.96	13.7	b
20	103.23	122.95	13.4	b
30	188.75	182.85	19.95	c

Table 11: Number of Chyronomid larvae at different salinities.

NaCl concentration (gr./l)	Mean	Std. Dev	Std. Error	Fishers protected $LSD_{0.05}$
20	1.67	6.93	0.75	a
30	6.64	57.4	6.2	a
10	8.6	55.86	6.09	a
0	80.84	106.23	11.6	b

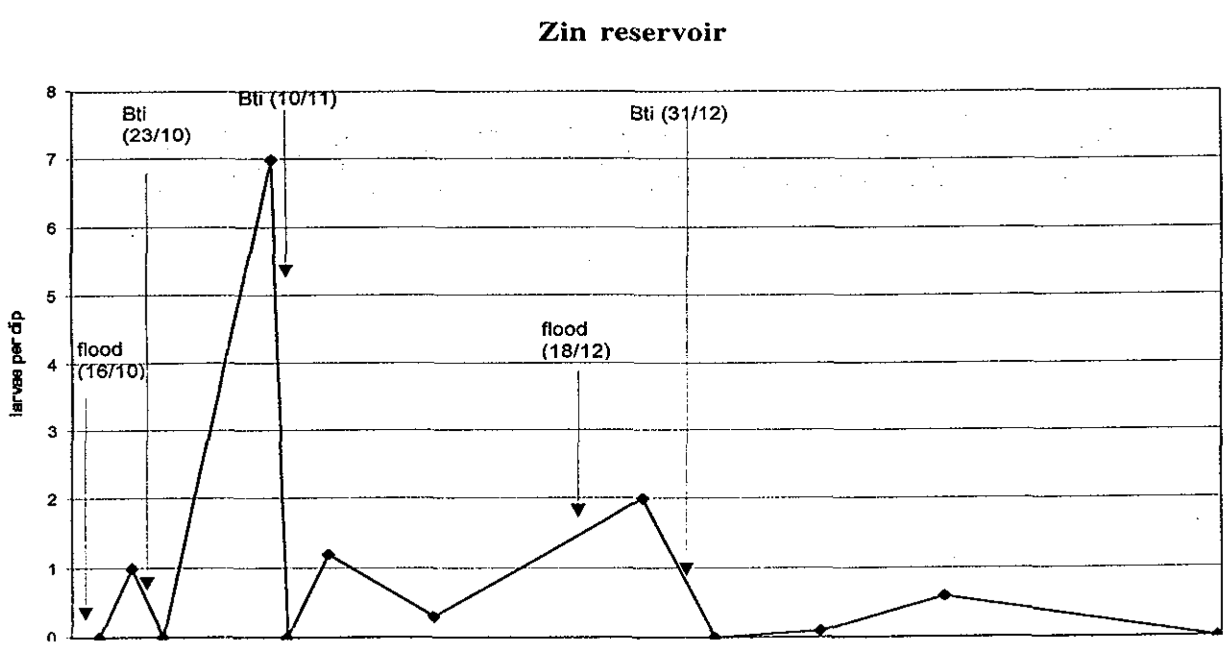
d. ***Integrated bio-control measures using Bacillus thuringiensis israelensis (Bti).***

Control measures were carried out during the current reporting period using a suspension concentrate formulation (SC) of Bti, Biotush (BioDalia). Biotush was applied at a rate of 5.00 kg/ha at a spray volume of 50 liters per hectare from a Bell Helicopter and also using knapsack sprayers in Zin reservoir. The biological activity of Biotush was tested in a bioassay to determine the dose-response curve. The protocol of this bioassay is described below.

Zin

The Zin reservoir was flooded twice during the current reporting period (fig. 18), 16/10 and 18/10/2000. The reservoir was sampled for the presence of mosquito larvae 2 days following the first flood, when no larvae were found and 4 days following the flood, when ca. 1 larvae per dip was recorded. Bti application was made immediately in order to prevent mass breeding of *Ae. caspius*, known to breed in this habitat. The treatment reduced the existing population to zero however developing eggs apparently hatched giving rise to an additional wave of larvae which was again reduced to zero by an additional Bti application. The second flood gave rise to a smaller population which was controlled with a spot treatment with Bti using a knapsack hand sprayer.

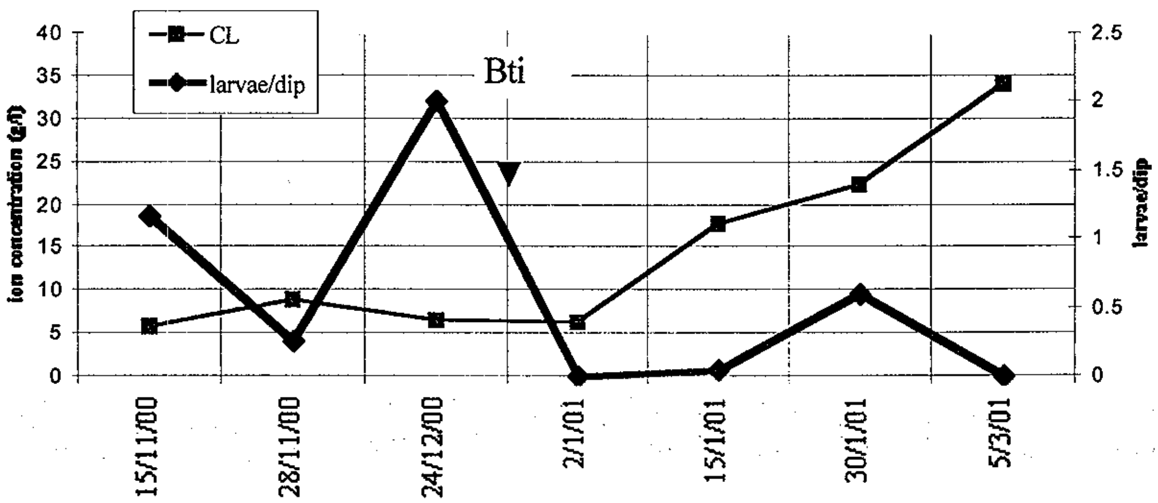
Figure 18. Biological control of mosquitoes in the Zin reservoir , Southern Dead Sea Basin, 2000



Relation between salinity and mosquito abundance in Zin reservoir.

Aedes caspius larvae were the dominant or the only species in Zin reservoir. Their populations increased even at ion concentrations greater than 20 g/l, decreasing at levels greater than 35 g/l (fig. 19).

Figure 19. Ion concentration fluctuations and larval abundance in Zin reservoir.



Heimar

The Heimar reservoir was also flooded in the spring of 2001 (4 May, 2001). Samples were taken ca 2 weeks following the flood and a significant population was found on the 25 May (table 12).

Table 12. Larval populations in the Heimar reservoir after flooding on 4 May 2001

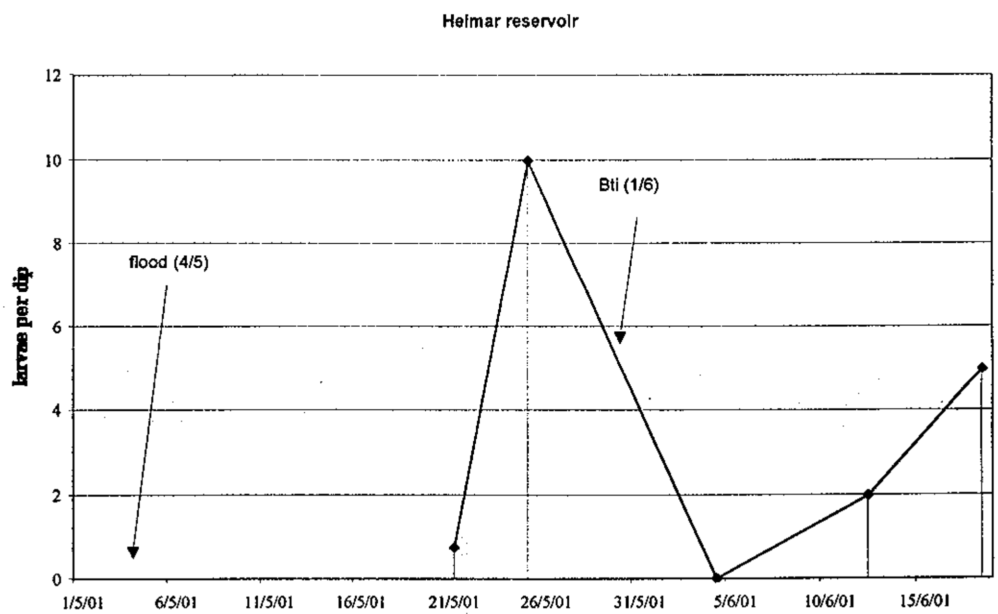
Heimar		
date	Larvae/dip	species
21/5/01	0.75	**table 12a**
25/5/01	10	**table 12b**
4/6/01	0	
12/6/01	2	*Cx. pusillus*
18/6/01	5	*Cx. pusillus*

Table 12. continued.

Table 12a	Aedes caspius	Cx.pusillus				
Total	4	5				
%	45	55				
Table 12b	*Cx. pipiens*	*Cx.pusillus*	*Perexiguus*	*Caspius*	*Culiseta*	total
Total	23	106	11	14	1	162
%	14.2	65.4	6.8	8.6	0.6	100.0

Treatment of this reservoir is more difficult than Zin due to heavy growth of tamarisk, reed and other vegetation. Cleaning operations carried out in the previous year enabled us to effectively conduct aerial application this year. A liquid suspension formulation of Bti, BioTush (BioDahlia) was used at a rate of 7.0 l/ha and a spray volume of 500 l/ha. This application effectively reduced the larval mosquito population to very low numbers and no repeat applications were necessary (fig. 20).

Figure 20. Biological control of mosquitoes in the Heimar reservoir, Southern Dead Sea Basin, 2000



As part of the IBC drain ditches were cleaned of heavy vegetation of Fragmites and Typha, enabling free flow of water and increased efficacy of the indigenous sal marsh minnow (killifish) *Aphanius dispar*. As a result no or few mosquito larvae were found as compared to the dense populations found before introduction of IBC.

III Transfer of technology and recommendations

a. Houseflies

During the course of this project we have collaborated with the local municipality and farmers. Some of our reports were translated in the Hebrew language, modified into manuals and distributed to all persons responsible for fly abatement, farmers and interested parties. Personnel of the municipality were instructed in biological aspects of flies and monitoring. The farmers collaborated with us in collection of agricultural waste and the process of composting including . Agrotechniques were improved by incorporating the compost into the soil prior to seeding and irrigating.

1. Solid agricultural waste management program
   Develop regional compost facility
   Develop waste collection system
2. Develop enriched compost with specification for individual crops and antagonists.
3. Agricultural practices
   Incorporation of fertilizers into soil
   Use mature compost rather than raw manure
   Good agricultural practice – maintain clean fields
4. Introduction of biological control agents
   In poultry farms release indigenous fly predator *Hydrotaea (Ophyra) aenescens*.
5. Fly population prediction model based on input from monitoring data
   Utilize distribution model to predict timing and extent of population outbreaks
6. Interdiction of adult populations
   Optimize location, density and timing of interdiction traps (maximum expected interdiction about 10% of adult population).
7. Municipal solid waste (MSW) program
   Improve collection, disposal and treatment.
8. Wastewater treatment facility modification to reduce fly breeding in exposed sludge.

b. Mosquitoes

The practices of integrated biological control (IBC) of mosquitoes based on utilization of all available techniques including: physical source reduction, utilization of the environmentally friendly biological control agent *Bacillus thuringiensis* subsp. *israelensis* (Bti) and augmentation of indigenous natural enemies (*Aphanius dispar, Anax imperator* and others), were transferred to personnel responsible for mosquito abatement, governmental agencies and all interested parties.

In the course of this study we have developed intervention methods pertinent to each of the larval mosquito habitats. The methodologies were transferred to the transferred to personnel responsible for mosquito abatement.

1) Salt marshes – No need to treat Dead Sea perimeter due to the increase in potassium and magnesium ion concentration, detrimental to aquatic organisms. Salt marshes further removed from the Dead Sea near the agricultural area occasionally generate local populations of *Ae. caspius* , *Cx. pusillus* and *A n. multicolor*. Our recommendation is to drain these seasonal localized marshes.
2) Drainage canals – In early stages of study, were overgrown with rooted vegetation and high densities of diverse mosquito populations. Through our intervention the canals were cleaned by heavy equipment such that local indigenous killifish could effectively control larval mosquito populations without any need for other control interventions including Bti.
3) Springs – Insignificant breeding source in which populations are checked by indigenous invertebrate predators and killifish. This information was transferred to the municipality so that no unwarranted treatments be made, resulting in environmental disturbance.
4) Sewage treatment pools and overflow canal – Constituted a breeding place for *Cs. pipiens*. We have recommended to treat it in several ways: a) by alternating sewage flow between two separate canals every 5 days, b) Clean canal of rooted vegetation.
5) Zin Reservoir – On rare occasions in the winter months, when the reservoir is flooded by rains, huge populations of *Ae. caspius* larvae hatch from eggs within a few hours. These populations should be treated by aerial spraying of Bti by helicopter within

less than one week from the first appearance of larvae. This water body becomes a breeding habitat for up to a dozen mosquito species, including the nuisance mosquito, *Cx. pipiens*, often resulting in the necessity to treat 2-4 aerial applications in one season. During the past year the local municipal personnel were versed on the application protocols and monitoring methods.

6) Heimar reservoir - Overgrown with mostly *Tamarix* and reed vegetation. We have recommended and implemented opening paths with heavy equipment so that previously inaccessible areas could be treated from a boat platform. Bti is the sole control agent used in the reservoirs. We have recommended also the introduction of killifish.

C. Scientific Impact

The findings of this joint project provides a scientific basis for developing an integrated biological control program both against fly and mosquito populations in the area.

Monitoring techniques of fly populations were developed and standardized jointly by all three teams and then utilized to determine seasonal fluctuations and spatial distributions of adult flies. These population censuses along with results obtained from the other experiments conducted during the 1997 enable us to determine the efficacy of interdiction traps in a comprehensive control program. Additional information regarding fly breeding potential in the various agricultural crops and refuse enables us to focus our future efforts on the primary contributors to the fly problem.

Results of mosquito population monitoring during the 1997 season emphasized the importance of routine surveillance, since a completely new and relatively extensive breeding site was discovered during the 1997 season in the Heimar reservoir. Determination of the efficacy of various Bti formulations under laboratory and field conditions was also carried out and protocols were fine tuned for transfer to private and public institutions involved with applying and producing Bti.

Findings during the 1997 season emphasize the importance of this joint effort in providing solutions to the subject problems. The ultimate goal of providing environmental solutions to regional problems has become an attainable goal within the near future and will provide the scientific basis for a comprehensive regional integrated biological control program.

D. Description of Project Impact

1) Environmental solutions to arthropod pest infestations in an extensive area bordering Israel, Jordan and Israel, Gaza Strip have been made a foreseeable goal through the cooperative efforts of all participants.
2) Cooperative research between neighboring countries in the Middle East has been shown in this joint project to be a feasible means of improving the standard of living and promoting economic development of all neighboring countries.

E. Strengthening of participating country institutions

The project strengthens the participating laboratories technologically, scientifically and administratively. New research tools have been developed to determine arthropod infestations and to deal with the problems of public health pests. These new techniques developed for the purpose of scientific investigation have also been transferred to local municipalities which are currently using the techniques developed in order to overcome the problems at hand.

F. Future Work

As a spin-off of this project , we have planned a project entitled "Environmental Management of Arthropod Pests of Public Health and Economic Importance" to develop and implement the solutions which we have determined over the past several years. This project will be submitted as a full proposal to USAID MERC in the next funding cycle.

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3.Technical project objectives:

1. Develop an early warning system for outbreaks of arthropod pest populations in the border regions of the Jordan Rift Valley and Gaza Strip utilizing remote sensing and GIS.
2. Develop an integrated biological control program for the suppression of nuisance house fly populations in the region, evaluating the feasibility of *Ophyra aenescens*, a predatory fly, against house fly larvae.
3. Investigate tolerance and adaptation of halophilic mosquito species to various ion concentrations and relationship of ion concentrations to control efficacy using Bti.
4. Develop a solid waste management system initially designed to recycle agricultural refuse and later to include all organic municipal solid waste (MSW), as part of the overall environmental solution to massive house fly outbreaks.

4. **Justification:** The regional economic goals of Israel and its neighboring Arab nations include development of tourism and agriculture in the context of development of the border regions of Israel/Gaza and Israel/Jordan. Economic development of these border regions is dependent both on progress of the peace process and solutions to the many environmental disturbances repressing economic growth. Improper solid waste management and faulty treatment of effluents are perceived as significant environmental concerns leading to uncontrolled pollution and outbreaks of arthropod pests which constitute a serious nuisance problem and act as potential vectors of human and animal diseases. A joint effort by Israel and its

neighboring Arab countries to solve these regional problems can contribute significantly both to the progress towards peace and to economic and social prosperity.

5. Technical Discussion:

a) **Summary of relevant background:** The Dead Sea Basin on the floor of the Jordan Rift Valley (JRV) at the Jordanian-Israeli border, and the Gaza Strip Region play important roles in the region's economy. Both the tourist industry and agriculture are key sources of income. The mild winters allow production of off-season, high-value vegetables and fruits marketed in Europe. Infestations of anthropophilic insects, such as mosquitoes and flies, are major pest problems and threaten human health. These periodically plague tourists and local residents of the region. From 1995-2000, in the framework of our joint project "Environmental Solutions to Arthropod Pests of Public Health Importance in the Jordan Rift Valley and Gaza Strip", recommendations were developed for an integrated biological pest management system to control these massive fly outbreaks. We also developed recommendations for integrated biological control (IBC) of mosquitoes. A reliable monitoring system provides a continual input of vital data for measuring the effect of interventions. Spatial and temporal distributions of fly and mosquito populations were estimated. Adult fly population dynamics were described by major fall peaks and minor spring peaks (appendix 1). Massive amounts of data have been analysed to provide a preliminary predictive model for seasonal fluctuations. Agricultural practices and seasonal flooding are the key driving components of this model for fly and mosquito populations, respectively.

Remote sensing: Since the remote sensing satellite Landsat-1 was launched in 1972, remotely sensed (RS) data have been used to map features on the earth's surface. An increasing number of health-related studies have used remotely sensed data for monitoring, surveillance, or risk mapping of vector-borne diseases (Beck et al., 2000; Dale et al., 1998; Hay, 1997; Hay et al, 1998). The special Health Applications Office (HAO) was established in the early 1970's by National Aeronautics and Space Administration (NASA) to investigate remote sensing applications to public health. Application of the low spatial resolution RS data has a significant advantage - very high temporal resolution (12 hours). These data are widely used for monitoring day-to-day dynamics of vegetation, land surface temperature, and atmospheric moisture at the global, continental, and regional scales. Dale and Morris (1995) developed a detailed methodology for estimating and predicting potential mosquito breeding areas using aerial photographs and GIS. Multi-seasonal analysis of Landsat-TM images for dry and wet season and calculation of NDVI values indicate potential mosquito habitats through water and vegetation characteristics (Pope et al., 1994; Rejmankova et al, 1991). These studies demonstrate the potential for risk monitoring and subsequent mosquito control efforts prior to disease outbreak. The remote sensing techniques proved to be cost-effectiveness in mosquito and fly control campaigns, because some satellite imagery is free of charge and could be obtained by the internet from NASA and NOAA data bases. Data from satellite imagery, aerial photography, statistics, and ground observations may be incorporated into a GIS database, which facilitates the storage and management of the data and enables multiple overlay of data on map sections. Increased computing power and GIS spatial modeling capabilities extend remote sensing use beyond the research community into operational disease surveillance and control.

House fly IPM: The house fly, *Musca domestica*, is a major economic and public health pest in and around animal production facilities and open agricultural fields. Known mainly as a nuisance pest, this fly has also been known to transmit many enteric and ophthalmic diseases of humans and animals (Harrington and Cohe1989, Greenberg 1973, Cohen et al. 1991; et al. 1988). Adult flies are known to disperse 20 km from their breeding sites (Bishopp and Laake 1921), thus affecting not only are the farmers and their families, but also their neighbors. We investigated fly dispersal in the Jordan Rift Valley and found that fly populations disperse p to 20 km from their release sites and travel at 8 km per hour (Margalith et al., unpub.). With increased awareness of environmental quality and regulations protecting the environment, there is additional pressure to find effective solutions to nuisance pest problems with minimal environmental impact. The black dump fly, *Hydrotaea aenescens*, is a recently introduced beneficial fly whose larvae feed on the larvae of house flies and other filth fly species. They can consume 17-20 house fly larvae per day (Hogsette 1979, Geden et al. 1988) and reduce or eliminate house fly populations on poultry facilities (Nolan and Kissam 1985; Turner and Carter 1990). The black dump fly is sold commercially as a biocontrol agent for house fly. Unlike house flies, adult black dump flies are reported to generally remain in manure pits where they breed (Turner and Carter 1990), with unnoticed dispersal to other farms (Hogsette and Jacobs 1999). Dump flies compete well with house flies, but prefer a more narrow manmoisture range (Farkas et al. 1998).

Tolerance and adaptation to salinity in halophilic mosquito species: Mosquito larvae are extremely adaptable to environmental variations, as evidenced by the presence of different species in a great diversity of aquatic habitats. They can be found in 0° C melt water pools in the Arctic to temporary desert pools reaching 50° C, in water qualities ranging from fresh spring to sewage and salty waters in coastal and desert environments. This adaptability induced by genetic plasticity has given them the advantage of successful reproduction in habitats with extreme environmental conditions where competition and predation pressure is reduced. As a rule *Culex pipiens* is a cosmopolitan fresh water species. Due to genetic plasticity it has adapted to extremely high salinity in the natural breeding sites around the Dead Sea. A few of the most infamous disease transmitting mosquitoes are known to breed in salt water swamps and brackish ponds along the ocean coasts. Some salinity tolerant species of the mosquito genera *Aedes* and *Culex* transmit a number of severe diseases including dengue fever and filariasis. Other examples of salt water tolerance are commonly found among the malaria vectors (e.g. *Anopheles multicolor, An. sundaicus, An. sacharovi, An. stephensi, An. gambiae.* Margalith & Tahori (1974) reported that *An. multicolor* and *Aedes caspius* were able to breed in 14% and 13% salinity, respectively. The Dead Sea Basin (DSB), part of the Jordan Rift Valley, is a populated region with agriculture and a rapidly growing tourism industry as the main source of income. The area has a high concentration of ions in the soil and rock deposits which cause high salinity in spring water pools and

drainage canals. This creates habitats for mosquitoes and other organisms with high tolerance or even prevalence to salinity. Some of the halophilic species mentioned above are indigenous to the area. Some parasites and pathogenic viruses may be carried here by vectors and hence transmitted to the people and domestic animals. Of the 16 mosquito species recorded in previous studies in this area, 5 are considered to be of potential public health importance: *An. sergenti, An. multicolor* and *An. superpictus* are malaria vectors (MERC 1995); *Cx. pipiens* and *Cx. perexiguus* are the vectors of West Nile fever, Sindbis fever and the deadly Rift Valley virus. The DSB is regularly monitored and breeding sites are controlled by fish (*Aphanius dispar*) and B.t.i. (*Bacillus thuringiensis israelensis*), a natural occurring bacteria pathogenic to mosquito larvae (Goldberg & Margalit, 1977).
Compost: During the growing season, large quantities of agricultural waste, e.g., unmarketable fruit, plant residues and other organic solid waste, creates a phytosanitary problem and a breeding habitat for house flies. Household and livestock refuse also accumulates. The proportion of organic material in agricultural and household waste is high enough to be recycled as compost. Municipalities are attempting to eliminate burial of solid waste because is expensive and not usually effective. Meanwhile, heavy fly infestations plague the area during the fall and spring seasons, invading tourist recreation areas, spas, and psoriasis health resorts along the Dead Sea Basin.

b) Technical Program Description:

1. **Develop an early warning system for outbreaks of arthropod pest populations in the border regions of the Jordan Rift Valley and Gaza Strip utilizing remote sensing and GIS.** Methodology to be used by the Remote Sensing Laboratory consists of the following: <u>i. Define environmental indicators</u> that can be observed by remote sensing to identify fly and mosquito breeding sites. Initially, historical events in the Dead Sea area will be analyzed. Information will be collected from different sources including literature, reports, field survey, and personal interview. The origin of the flies and mosquitoes and their trajectories will be studied. In addition, environmental conditions such as rainfall, vegetation, wind direction, soil and air temperatures, and soil moisture will be simulated. Such information is available from different sources in Israel and Jordan, and other international sources (NASA databases, WMO, and more); <u>ii. Day-to-day monitoring and image analysis</u> using NOAA/AVHRR and SeaWiFS images in HRPT format 1 km spatial resolution acquired day by day by the satellite receiving station located at Sede Boker, which covers the whole region (Figure 1). This station provides several types of indicators: (1) Vegetation indices (such as the Normalized Different Vegetation Index, NDVI)) for assessing vegetation state and condition, phenology, green coverage etc.; (2) Land surface temperature; and (3) Open water. Images will be subject to radiometric, atmospheric and geometric corrections, using procedures developed in the Remote Sensing Laboratory. The final product(s) of this phase will be coarse spatial resolution but high temporal resolution images that show spatial and temporal distributions of the different indicators defined in Phase One. These data will serve as a false alarm before moving to the next detailed phase; <u>iii.</u>

High spatial resolution image analysis to tune and scale down the products produced in Phase Two. For this phase, images will be used from the Landsat-ETM+ sensor (Figure 2). This sensor is characterized by high (30 m) spatial resolution but relatively low temporal resolution of 16 days. Since the Landsat data are complementary information to the NOAA data, the former images will be analyzed for any suspected indicator that had been received earlier by the NOAA data analysis. The Landsat data also will be subject to radiometric, atmospheric, and geometric corrections. The most basic product from this processing will be a vegetation index, but others (such open water bodies) can also be created. After geometric correction the image and/or its product will be integrated in the GIS of the next phase; iv. GIS implementation to locate potential fly and mosquito breeding habitats. This will be based on digitized maps of the topography, rainfall, vegetation, soil, hydrography, water bodies, as well as the Landsat images and their products. These types of maps are available in different scales and projections. By overlaying these GIS layers, a prediction model showing variation of possibilities for the insects' origin and breeding will be produced.

2. **Develop an integrated biological control program for the suppression of nuisance house fly populations in the region, evaluating the feasibility of *Ophyra aenescens*, a predatory fly, against house fly larvae.**

i. Preliminary field survey to determine naturally occurring predator and pest population levels and their population dynamics. Three to four typical caged-layer poultry farms will be chosen for the sampling program. Poultry houses will be similar in design and there must be evidence that both *M. domestica* and *H. aenescens* are present on site. Air temperature inside the houses, measured either with maximum-minimum or portable digital thermometers, will be recorded when fly samples are collected. **Adult Flies** of both species can best be sampled using the sticky card method (Hogsette et al. 1993; Hogsette and Jacobs 1999) Many methods exist for sampling house fly adults, but no traps or baits have been developed for use with *H. aenescens*. Cards will be placed on support posts ca. 67 cm above the ground at the rate of 1 card per 10 meters. For example, a poultry house that is 100 m in length will receive 10 cards spaced at regular intervals. Cards will be replaced at weekly intervals and flies trapped on exposed cards will be counted by species. **Immature Flies (Pupae)** will be collected by digging samples from manure in poultry houses. Pupae will be separated from the manure and the first 250 intact pupae will constitute a sample. Pupae will be collected from each house weekly, and the fly species determined by inspection of posterior spiracles. **Manure Samples** will be collected at least monthly (ten 100-mg samples per house) at fly collection sites to determine moisture ranges used by the flies. Manure samples will be taken from sites where larvae are present, placed individually into plastic bags, sealed, and returned to the lab on ice. will be weighed, heated at 50 C for 48 hours and re-weighed to determine percent moisture. ii. Laboratory rearing - Although there are many techniques in the literature for laboratory rearing of *H. aenescens* (e.g. Hogsette and Washington 1995), a representative diet must be developed from constituents available commercially in Israel and flies must be reared at moisture levels consistent with those found in fly survey studies above. **Initial Studies:** Candidate diets consisting of locally used house fly diets plus a protein source, such as fish meal, meat and bone meal, or feather meal, will be mixed at various concentrations and moisture ranges. These diets will be seeded with eggs collected from wild *H. aenescens* populations. Measures of diet suitability will include percent survival, pupal weight, adult weight, and development time. Control (standard) measurements will be based on those found in the literature. Based on fly performance, a diet will be selected to use for laboratory rearing. **Colonization of Flies:** *H. aenescens* will be colonized in the laboratory using the selected diet and techniques developed by Hogsette and Washington (1995). Adaptation of *H. aenescens* to laboratory conditions can take much longer than expected for other flies, such as *M. domestica.* A protein source must be included in adult diets, at least initially, to ensure maximum egg production. Colonies from other countries could be purchased to save time, however it is doubtful whether flies from commercial colonies in the U.S. or Nothern Europe could withstand local conditions in Israel if released in the field. iii. Laboratory experimentation to determine predatory capacity of local *H. aenescens* strains, i.e. the **functional response** = increased natural enemy consumption of pest species prey in response to increases in pest population density. Known numbers of second-instar *H. aenescens* larvae will be placed in 150-ml beakers containing a larval medium that has no nutritional value. This forces larvae to feed on house fly larvae which will be added to the beakers in predetermined numbers on a daily basis. Treatment levels will consist of constant numbers of *H. aenescens* and increasing numbers of house flies. Controls will consist of beakers with only house fly larvae or only *H. aenescens* larvae. iv. Mass culture - Design mass-rearing methods for production of *H. aenescens.* Published mass-rearing techniques (Hogsette and Washington 1995) will be adapted to BGU facilities. Small laboratory rearing containers will be replaced by large (60 x 90 x 10 cm) pans suitable for rearing 30 to 60 thousand individuals. Time may be required for *H. aenescens* to adapt to rearing conditions in the large pans. Eggs collected from the colony will be washed and measured (1 cc of eggs = ca. 10,000 eggs) so the optimum number of eggs can be added to pans of medium. If 2nd-instar larvae are to be released in the field, 60,000 eggs can be added to a pan of medium because larvae be released before their development has been completed. If pupae are to be released, pans can only be seeded with 30,000 eggs. Pupae and adults will be weighed and development time monitored to be sure that performance of flies in the mass rearing facility remains similar to that of flies in the smaller laboratory colony. v. Field releases - *H*. aenescens larvae and/or pupae will be released weekly during 3 tests over a 14-month period at farms where *H. aenescens* is not present or present only in small numbers.. No *H. aenescens* will be released at control farms. Purpose of the tests will be to determine whether *H. aenescens* can become established and become the dominant fly species. Releases will be made weekly at the rate of 300 individuals per linear meter of manure in one row in each house. Larvae will be spread down the rows of manure by hand and pupae will be placed in release stations attached to support posts in houses. Treatments will incude: 1. Single, innoculative releases, 2. Weekly releases made until *H. aenescens* populations are roughly equal to house fly populations based on sticky card counts. vi. Field evaluation to rate the success of mass releases under field conditions. Flies (adults and pupae) will be monitored as described in the survey project above. Observations will be made of manure conditions and behavior of adult flies, particularly *H. aenescens*. Sites adjacent to release sites will be monitored for presence of *H. aenescens* adults as an indication of dispersal. Opinions of poultry producers about efficacy of *H. aenescens* will also be solicited.

3. **To investigate tolerance and adaptation of halophilic mosquito species to various salt ion concentrations and their relation to control efficacy using Bti.** Tolerance to ion concentrations will be investigated by: a) monitoring natural breeding sites for salinity levels and species (*Aedes caspius, Culex pusillus* and *Culex pipiens*) composition and abundance, b) field testing oviposition preference and larval development in artificial pools with varying ion concentrations. Adaptation to changes in ion concentration will be investigated under laboratory conditions by determining the existence of ionic and osmotic regulation in mosquito larvae haemolymph. Ion and amino acid concentrations in the haemolymph will be related to increasing ion concentrations for *Ae. caspius, Cx. pusillus* and *Cx. pipiens* collected from the Dead Sea region.

4. To develop a solid waste management system initially designed to recycle solid organic agricultural waste and later to include all municipal solid waste (MSW), as part of the overall environmental solution to massive housefly outbreaks.

This part of the project will be carried out in four phases:

I - Baseline data
II - Collection system
III - Compost process
IV - Redistribution into agricultural fields

Phase I – Baseline data

In order to measure the success of this project several parameters will be monitored throughout its duration. These parameters include: fly activity level, phytosanitary conditions, landfill volume and area, groundwater quality and compost quality and quantity. In many cases, protocol have been established for making reliable measurements of the relevant parameters, however in some cases, methodologies must be developed or fine tuned for the conditions prevailing in the region.

Phase II – Collection system

The collection system will be divided into three major components:

1) collection of agricultural solid organic waste (ASOW) from the field
2) transport of ASOW to the compost facility.
3) transport of animal manure from farms to compost facility

Phase III – Compost process

This phase consists of recycling solid organic agricultural waste into enriched compost. In order to develop a successful compost process the following steps are critical: waste stream investigation, site location, determining facility requirements, pilot installation construction, composting (two compost processes will be tested simultaneously: thermophilic and vermicomposting) and quality control.

Composting Composting is the result of a dynamic, biological process of decomposition and stabilization of organic wastes or substrates. The desired end products are a stable, mature, and high quality compost which can be used for soil conditioning and fertilization.

Thermophilic composting: The windrow composting method, the most widely used, will be adapted in this project. Agricultural wastes such as animal manures, plant residues, crop residues, etc. will be arranged into windrows and mechanically turned at regular intervals to enhance environmental conditions for microbial activity and decomposition. Moisture content of the decomposing organic waste will be maintained at 45-60% RH. The daily wet weight, fractional solids contents, and the fractional volatile solids content of the organic waste of the compost facility's mass balance will be monitored; as well as oxygen levels, organic material particle size, nutrient levels, the carbon-to-nitrogen ratio, moisture content, temperature, and pH.

Vermicomposting – Composting with earthworm *Einsenia fetida* - The most commonly used processing method for vermicomposting (vermicompost) is a rick. A rick is a windrow that contains agricultural wastes such as animal manures, plant residues, crop residues, etc. Fresh organic waste is cyclically layered in 7-12 cm (3-

4 inches) layers on top of the rick once or twice a week, depending on the amount of available feedstock, season, and weather. After repeated applications of organic wastes, the overall accumulated zone of decomposition is the top 15-25 cm (6-8 inches) of the rick. A 70-80% moisture content of the decomposing organic waste is maintained. Vermicomposting operations will have a mist system laid on top of the ricks to maintain the top 15-20 cm(6-8 inches) portion of the ricks at 70-80% moisture content. Moistening will be carried out for several short cycles (5-8 minutes) as required under the prevailing conditions. To enhance earthworm activity, a shaded area for the vermicomposting operation will be installed. The option of using mixtures of selected microorganisms to enhance the decomposition of manure components (e.g., uric acid) and plant fiber (e.g., cellulose) is a possibility. Or some composting organic matter that is high in microbial activity and populations can be "seeded" as an inoculum onto fresh arriving organic matter to hasten the rate of decomposition if desired.

Compost quality control parameters; Quality control of compost during and after the compost process will be based on measurement of chemical, biological and physical parameters. This aspect of the project is discussed under Phase I, baseline data.

Phase IV – Redistribution (recyling into agriculture)

Transportation and distribution of the compost products to grower fields.

The final compost product will be brought to the fields from the compost facility.

Incorporation into the field at different rates according to crop.

Trials will be carried out to determine optimum application rates for the main crops grown in the region.

The effect of compost on soil-borne diseases (see baseline data) and on yield and product quality.

Trials will be carried out to determine the effect of compost on crop yields.

Soil-borne pathogens. The presence of soil-borne pathogens will be determined in a sampling program to be carried out throughout the course of this study.

6. **Innovation.** Highly evolved remote sensing and GIS will be implemented to develop an advanced warning system for pest outbreaks. Data collected on both sides of the political borders will for the first time be collated and analyzed as a single entity. Appropriate compost technology adapted for the prevailing conditions in the region will be developed and implemented.

7. **Project Structure and Management** (appendix 2). The PI (Jerome Hogsette, USDA, Gainesville, Florida) will be responsible for overall project management including receipt and distribution of funds to the participating institutions . Experts in each of the four fields of research (appendix 3): 1) remote sensing, 2) biological control of flies, 3) mosquito ecology and 4) composting solid organic waste, will plan the activities under his/her expertise. Teams, headed by each of the research directors in their respective institutions, will responsible for carrying out the plans. Results will be collated into one database and analysed. Periodic meetings and workshops will be conducted to transfer information and technology and to focus future activities. The final product, Management System will be constructed under

the PI's direction by collating all results from each of the main research fields and further activities will be recommended for fine tuning the system. The U.S. partner is involved because his level of muscoid fly expertise is not available in the Middle East.

8. **Arab-Israeli Collaboration.** The participating research team has had years of fruitful scientific cooperation under the preceding project "Environmental Solutions to Arthropod Pests of Public Health Importance in the Jordan Rift Valley and Gaza Strip" under framework of MERC. This project is a natural continuation of its predecessor both in terms of applicative solutions to the pressing problems and building ties between neighboring countries. Collaboration will be based on an open channel of communication and complete exchange of research results which will be analyzed and used to continually update the work activities of all participating research teams.

9. **Expected Benefits:** This project will potentially provide benefits for the inhabitants of the region by providing an early warning system for incumbent pest outbreaks, introduce a viable biological control program thus maintain a biologically diverse ecosystem, provide vital information necessary for controlling larval mosquitoes in saline environments and establish a compost plant for recycling agricultural organic waste. Reduction of pest populations using environmentally friendly means will increase the value of land, improve attractiveness for tourist sites and improve the quality of life. The compost project will in addition provide farmers with enriched high quality compost for high yield crops and improve the soil structure of the nutrient poor natural soils existing today. Addition of antagonists will also provide an alternative to methyl bromide treatments, to be banned by year 2002.